TOXICOLOGICAL EVALUATION
OF A TREATED MUNICIPAL EFFLUENT
BIOMONITORING SUPPORT FOR A NPDES PERMIT:
July 2016

Warner Village Wastewater Treatment Facility
Warner, New Hampshire
NPDES Permit Number NH0100498

Prepared For:

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EXECUTIVE SUMMARY

The following summarizes the results of 48 hour acute exposure bioassays performed during July 2016 to support the NPDES biomonitoring requirements of the Warner Village, New Hampshire Wastewater Treatment Facility. Acute assays were completed using the freshwater species, *Ceriodaphnia dubia* and *Pimephales promelas*.

C. dubia, cultured at ESI, were <24 hours old juveniles released within 8 hours of one another. *P. promelas* were 9 days old at the start of the test. Dilution water was receiving water collected from the Warner River upstream of the discharge. Samples were received under chain of custody in good order. All sample receipt, test conditions and control endpoints were within protocol specifications, except where otherwise noted.

The results presented in this report relate only to the samples described on the chain(s) of custody and sample receipt log(s), and are intended to be used only by the submitter. Results from the acute exposure assays and their relationship to permit limits are summarized in the following matrix.

Acute Toxicity Evaluation

Species	Exposure	LC-50	A-NOEC	Permit Limit (LC-50)	Effluent Meets Permit Limit	Assay Meets Protocol Limits
Ceriodaphnia dubia	48 Hours	>100%	NC	100%	Yes	Yes
Pimephales promelas	48 Hours	>100%	NC	100%	Yes	Yes

COMMENTS:
NC = Not Calculated.

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1.0 INTRODUCTION

This report presents the results of toxicity tests completed on a composite effluent sample collected from the Warner Village, New Hampshire Wastewater Treatment Facility (Warner Village WWTF). Testing was based on programs and protocols developed by the US EPA (2002), with exceptions as noted by US EPA Region I (2011), and involved conducting 48 hour acute toxicity tests with the freshwater species, *Ceriodaphnia dubia* and *Pimephales promelas*. Testing was performed at EnviroSystems, Incorporated (ESI), Hampton, New Hampshire in accordance with the provisions of TNI Standards (2009).

Acute toxicity tests involve preparing a series of concentrations by diluting effluent with control water. Groups of test animals are exposed to each concentration and a control for a specified period. In acute tests, mortality data for each concentration are used to calculate (by regression) the median lethal concentration, or LC-50, defined as the effluent concentration that kills half of the test animals. Samples with high LC-50 values are less likely to cause significant environmental impacts. The acute no observed effect concentration (A-NOEC) provides information on the effluent concentration having minimal acute effects in the environment and is defined as the highest tested effluent concentration that causes no significant mortality.

2.0 MATERIALS AND METHODS

2.1 General Methods

Toxicological and analytical protocols used in this program follow procedures primarily designed to provide standard approaches for the evaluation of toxicological effects of discharges on aquatic organisms (US EPA 2002), and for the analysis of water samples (APHA 2012). See Section 4.0 for a list of references.

2.2 Test Species

C. dubia were maintained in laboratory water at 25±1°C with a photoperiod of 16:8 hours light:dark. Cultures are fed daily with a yeast/trout chow/Cerophyll or alfalfa leaves (YTC) mixture supplemented with *Pseudokirchneriella subcapitata* (algae) (US EPA 2002). Adults on a brood board were isolated 24 hours prior to test start and allowed to reproduce for 8 hours.

P. promelas were acclimated to approximate test conditions prior to use in the assay. Organisms were transferred to test chambers using an inverted glass pipette, minimizing the amount of water added to test solutions. Cultures were fed newly hatched *Artemia* nauplii until test start. Twenty control fish were weighed during the test to confirm loading rates. The loading rate was below the maximum 0.4 g/L recommended for assays conducted at 25°C. Fish weights and loading calculations are included in the data appendix.

2.3 Effluent, Receiving Water and Laboratory Water

Effluent and receiving water collection information is provided in Table 1. Samples were received at 0-6°C as per 40 CFR §136.3 unless otherwise noted, stored at 4±2°C and warmed to 25±1°C prior to preparing test solutions. Laboratory water was synthetic reconstituted water prepared at ESI according to protocol (US EPA 2002). This water has been used to successfully culture freshwater organisms since 1992.

Total residual chlorine (TRC) was measured by amperometric titration (MDL 0.02 mg/L) in the effluent sample. Samples with ≥0.02 mg/L TRC were dechlorinated using sodium thiosulfate (US EPA 2002).

2.4 Acute Exposure Bioassays

The 48 hour static acute assays were conducted at 25±1°C with a photoperiod of 16:8 hours light:dark. Test concentrations were 100% (undiluted), 50%, 25%, 12.5%, and 6.25% effluent. Daphnids were maintained in 30 mL test chambers with approximately 25 mL of test solution in each of 4 replicates with 5 organisms/replicate. Replicates in the *C. dubia* assay were not randomized; rather, test organisms were derived from a pool of mixed organisms recovered from ESI's culture the morning of testing. All organisms used were recovered from the same type of culture water. Minnows were maintained in 250 mL glass beakers with 200 mL of test solution in each of 2 replicates with 10 organisms/replicate. Replicates were not randomized during testing; rather, organisms were added randomly at test initiation by replicate across test solutions in an alternating fashion (alternating allocation).

Survival was recorded daily in all test replicates of both assays. A fifth replicate in the daphnid assay was included as a surrogate test chamber to obtain daily water qualities without disturbing the test animals, and was treated the same as actual test chambers with the addition of animals and food, but was not used to determine endpoint data. Dissolved oxygen and pH were measured daily, and specific conductivity was measured at the start of the daphnid assay. Dissolved oxygen was measured daily in all replicates and pH was measured daily in one replicate of each minnow test treatment; temperature was measured daily in one replicate of the laboratory water control. Specific conductivity was measured in one replicate of each test concentration at the start of the minnow assay.

2.5 Data Analysis

Data analysis involved, as required, determination of LC-50 values using CETIS™ v1.9.2.4, Comprehensive Environmental Toxicity Information System, software. The program computes LC-50 values using the Spearman-Karber and Probit methods following protocol guidelines. If survival in the highest test concentration was >50%, LC-50 values were obtained by direct observation of the raw data. As needed, the A-NOEC was determined as the highest test concentration that caused no significant mortality.

2.6 Quality Control

As part of the laboratory quality control program, reference toxicant evaluations are completed on a regular basis for each test species. These results provide relative health and response data and allow for comparison with historic data sets. See Table 2 for details.

3.0 RESULTS AND DISCUSSION

Results of the acute toxicity tests completed using *C. dubia* and *P. promelas* are summarized in Table 3. Table 4 contains effluent and diluent characteristics. US EPA Region I Attachment F toxicity test summary sheets are included after the tables. Support data, including copies of laboratory bench sheets, are provided in Appendix A.

Minimum test acceptability criteria require ≥90% survival in the control concentrations. Achievement of these results indicates that healthy test organisms were used and that the dilution water had no significant adverse impact on the outcome of the assay. See the Executive Summary and Table 3 for test acceptability.

4.0 LITERATURE CITED

40 CFR §136.3. *Code of Federal Regulations* (CFR), Protection of the Environment (Title 40), Guidelines Establishing Test Procedures for the Analysis of Pollutants (Part 136), Identification of Test Procedures (sub-part 3), Table II-Required Containers, Preservation Techniques, and Holding Times.

APHA. 2012. *Standard Methods for the Examination of Water and Wastewater*, 22nd Edition. Washington D.C.

The NELAC Institute (TNI). 2009. *Environmental Laboratory Sector, Volume 1: Management and Technical Requirements for Laboratories Performing Environmental Analysis (TNI Standard)*. EL-V1-2009.

US EPA. 2002. *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms.* Fifth Edition. EPA-821-R-02-012.

US EPA Region I. 2011. *US EPA Region 1 Freshwater Acute Toxicity Test Procedure and Protocol.* US EPA Region I Office, Boston, Massachusetts. February 28, 2011.

TABLE 1. Summary of Sample Collection Information.
Warner Village WWTF Effluent Biomonitoring Evaluation. July 2016.

Sample Description	Type	Collection Date	Collection Time	Receipt Date	Receipt Time	Arrival Temp °C
Effluent	Comp	07/05-06/16	0830-0725	07/06/16	1120	13 [a]
Receiving Water	Grab	07/05/16	0920	07/06/16	1120	13 [a]

COMMENTS:
[a] Upon receipt, the temperature was outside of the range of 0-6°C per 40 CFR §136.3 for NPDES effluent samples and support chemistry samples. Samples were received with ice in the cooler.

TABLE 2. Summary of Reference Toxicant Data.
Warner Village WWTF Effluent Biomonitoring Evaluation. July 2016.

Date	Endpoint		Value	Historic Mean/ Central Tendency	Acceptable Range	Reference Toxicant
C. dubia						
07/26/16	Survival	LC-50	44.2 [a]	22.7	2.6 - 42.9	SDS (mg/L)
P. promelas						
08/30/16	Survival	LC-50	39.4	32.6	24.0 - 41.1	SDS (mg/L)

Means and Acceptable Ranges based on the most recent 20 reference toxicant assays.
[a] Normal Acceptance Limits set at ±2 Std Dev of historic mean; maximum limits are ±3 Std of historic mean. The ±3 limit is acceptable, but considered high. If ±3 limit is utilized value is noted.

TABLE 3. Summary of Acute Evaluation Results.
Warner Village WWTF Effluent Biomonitoring Evaluation. July 2016.

Percent Survival

Species	Exposure	Lab	RW	6.25%	12.5%	25%	50%	100%
C. dubia	48 hours	100%	100%	100%	100%	100%	100%	100%
P. promelas	48 hours	90%	90%	90%	95%	90%	100%	100%

LC-50 and A-NOEC Results

Species	Exposure	Spearman-Karber	Probit	Direct Observation	A-NOEC
C. dubia	48 Hours	NC	NC	>100%	NC
P. promelas	48 Hours	NC	NC	>100%	NC

COMMENTS:
RW = Receiving Water; used as the diluent.
NC = Not Calculated.

TABLE 4. Summary of Effluent and Diluent Characteristics.
Warner Village WWTF Effluent Biomonitoring Evaluation. July 2016.

PARAMETER	UNIT	EFFLUENT	RECEIVING WATER
Specific Conductivity	µmhos/cm	610	109
pH	SU	7.01	6.78
Total Residual Chlorine	mg/L	<0.02	-
Alkalinity	mg/L	95	12
Hardness	mg/L	110	14
Total Solids	mg/L	430	94
Total Suspended Solids	mg/L	1.6	1.2
Total Dissolved Solids	mg/L	410	110
Ammonia	mg/L	<0.1	<0.1
Total Organic Carbon	mg/L	7.1	3.6
Aluminum, Total	mg/L	<0.02	0.047
Cadmium, Total	mg/L	<0.0005	<0.0005
Calcium, Total	mg/L	26	4.4
Chromium, Total	mg/L	<0.002	<0.002
Copper, Total	mg/L	0.036	0.003
Lead, Total	mg/L	0.00008	<0.0005
Magnesium, Total	mg/L	11	0.69
Nickel, Total	mg/L	<0.002	<0.002
Zinc, Total	mg/L	0.13	0.005

COMMENTS:
Additional water quality and chemistry support data are provided in Appendix A.